Supplementary Online Content

Lai L, Davey R, Beck A, et al. Emergency postexposure vaccination with vesicular stomatitis virus vectored ebola vaccine after needle stick. *JAMA*. doi:10.1001/jama.2015.1995

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Ebola Virus (EBOV) Antibody Enzyme-Linked Immunosorbent Assay (ELISA). Levels of EBOV-specific binding antibodies in serum were measured by indirect antigen-capture ELISA using recombinant EBOV glycoprotein (GP; Mayinga strain) minus the trans-membrane region (IBT Bioservices, Cat# 0501-015) as the antigen target. Antibody response against Ebola VP40 matrix protein was evaluated in a similar ELISA using recombinant EBOV VP40 matrix protein produced by a recombinant baculovirus in insect cells.[1] End-point titers of specific IgM or IgG were calculated as the most dilute serum concentration that gave an optical density reading of >0.2 above background.[2]

T Cell and B Cell Assays. Cryopreserved PBMCs were evaluated with multicolor flow cytometry for phenotyping of activated T cells and plasmablasts (antibody-secreting cells [ASCs]). Activated T cells were defined by co-expression of CD38 and HLA-DR on $CD4^+$ or $CD8^+$ T cells, separately.[3,4] Total B lymphocytes were gated as $CD3^-$/ $CD20^+$/$CD19^+$ cells and plasmablasts are plotted as the percentage of $CD27^{hi}$/$CD38^{hi}$ cells among total B lymphocytes. The ASCs are $CD3^-$/$CD20^{-/low}$/ $CD19^+$/$CD27^{hi}$/$CD38^{hi}$ cells[5]. The gating strategy utilized for identification of activated T cells and plasmablasts is provided in Supplemental Figure S1. Peripheral blood antigen-specific T cell responses were measured by intracellular cytokine staining using 15-mer peptides spanning the open reading frames for the gene encoding Zaire EBOV GP (Kikwit strain) to stimulate PBMCs overnight. The VSVΔG-ZEBOV vaccine encodes the Zaire EBOV GP from the Kikwit strain. Cytokine-positive cells were defined as a percentage within $CD4^+$ or $CD8^+$ T cells that produced IFN-□ ,IL-2, and/or TNF-α.[6,7.]

Real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. Zaire

Ebola virus glycoprotein (GP) RNA was detected with the EZ1 rRT-PCR; and Ebola nucleoprotein (NP) RNA was detected with an NP-specific rRT-PCR using the methods as previously described [8,9]. rVSV detection was done by rRT-PCR targeting VSV nucleoprotein (N) based on a previously published protocol[9] using a different primer set (forward primer: CGGAGGATTGACGACTAATGC; reverse primer: CGAGCCATTCGACCACATC). These assays were performed using Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument or ABI 7500 Real-Time PCR Instrument (Thermo Fisher Scientific Inc., Grand Island, NY) according to manufacturers' operating instructions and assay conditions described in the original literature. The EBOV GP assay is approved by the US Food and Drug Administration (FDA) under an Emergency Use Authorization (EUA).[10]

Detection of Plasma Cytokines. Human 63-plex cytokine kits were purchased from eBiosciences/Affymetrix for Luminex assays and used according to the manufacturer's recommendations with modifications. Beads were added to a 96-well plate and washed in a Biotek ELx405 washer. Samples were added to the plate containing the mixed antibody-linked beads and incubated at room temperature for 1 hour followed by overnight incubation at 4°C. Cold and room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Following the overnight incubation plates were washed in a Biotek ELx405 washer and then biotinylated detection antibody added for 75 minutes at room temperature with shaking. Plates were washed again as above and streptavidin-PE was added. After incubation for 30 minutes at room temperature, wash was performed as above and reading buffer was added to the wells. Each sample was measured in duplicate. Plates were read using a Luminex 200 instrument with a lower bound of 50 beads per sample per cytokine. Custom assay Control beads by Radix Biosolutions were added to all wells. The heat-map of the fold

change of cytokines, chemokines and growth factors in plasma was constructed in Excel using a 3-color scale (Microsoft Office 2011).

eReferences

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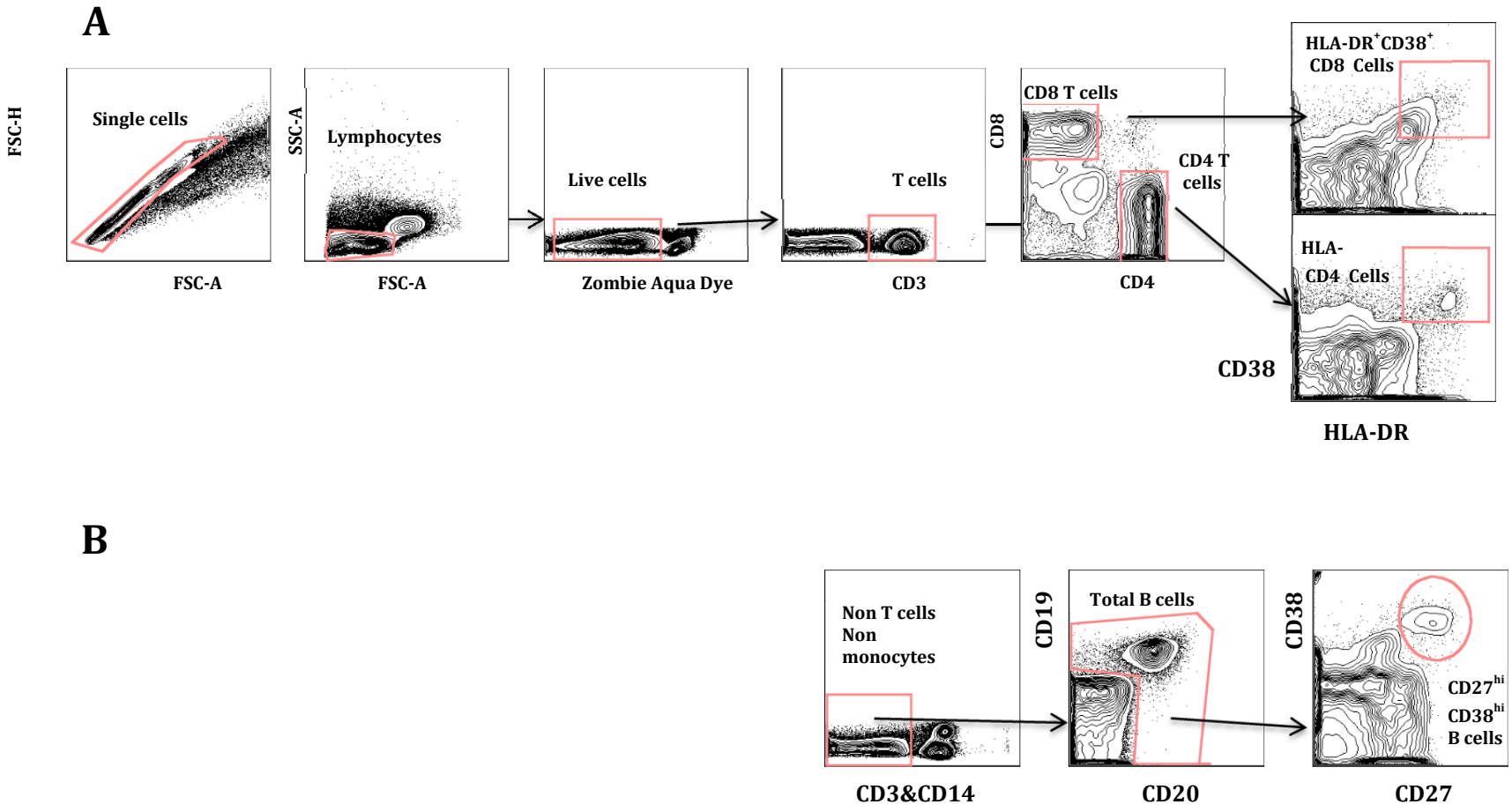
eTable. Absolute Levels for 63 Plasma Cytokines, Chemokines, and Growth Factors

Cytokine	Day	2	4	9	17	34	Irrelevant Control Plasma
IL-17F		1.48	1.04	1.08	1.52	1.27	1.14
sFAS		<2.31	<2.31	<2.31	<2.31	<2.31	18.59
TGF-a		3.76	1.42	1.13	1.5	1.11	7.17
MIP-1a		13.55	1.09	0.73	2.44	<0.60	12.53
SDF-1a		258.03	125.32	131.13	159.01	181.04	330.84
IL-27		65.89	15.65	6.94	12.48	<3.09	80.89
LIF		12.77	7.27	7.04	10	5.99	10.06
IL-1b		3.31	1.34	1.16	1.47	0.27	3.86
IL-2		30.99	19.6	18.58	24.37	16.84	33.13
IL-4		36.82	16.37	16.59	18.56	14.25	34.04
IL-5		<0.96	<0.96	<0.96	<0.96	<0.96	<0.96
IP-10		254.06	28.53	19.56	14.22	6.06	23.78
IL-6		<0.55	<0.55	<0.55	<0.55	<0.55	53.5
IL-7		7.42	7.45	5.23	2.61	2.34	4.19
IL-8		<0.12	<0.12	<0.12	<0.12	<0.12	0.7
IL-10		7.27	4.22	3.89	5.1	2.11	6.77
PIGF-1		<1.06	<1.06	<1.06	<1.06	<1.06	5.4
IFN-b		36.36	19.94	19.17	28.23	21.97	24.66
EOTAXIN		102.14	53.97	59.93	47.48	57.57	21.78
IL-12P70		1.69	0.93	0.85	0.77	0.58	1.92
IL-13		1.89	<0.08	<0.08	<0.08	<0.08	3.83
IL-17A		9.62	4.97	4.63	5.85	1.97	9.88
IL-31		<18.38	<18.38	<18.38	<18.38	<18.38	64.57
IL-1RA		3451.25	<37.62	<37.62	<37.62	<37.62	<37.62
SCF		5.08	4.34	4.34	3.58	3.89	14.84
RANTES		63.62	48.83	52.58	25.34	15.68	151.83
IFN-g		18.56	9.6	9.61	9.45	5.91	14.93
GM-CSF		2158.4	921.05	817.32	716.56	521.02	1512.58
TNF-a		77.16	75.22	75.56	78.92	83.44	75.39
HGF		199.41	110.35	106.27	127.32	102.35	107.91
MIP-1b		79.38	<2.86	<2.86	3.41	<2.86	102.06
IFN-a		16	4.66	4.66	4.19	2.47	8.24
TGF-b		6.15	1.81	1.54	0.84	<0.25	7.57
MCP-1		11.6	3.12	2.2	1.77	1.96	2.47
IL-9		<5.65	<5.65	<5.65	<5.65	<5.65	<5.65

VEGF-D		2.23	1.31	1.32	0.44	<0.28	10.33
TNF-b		<0.38	<0.38	<0.38	<0.38	<0.38	<0.38
bNGF		19.95	12.58	11.26	15.63	6.53	19.37
EGF		1.51	0.56	0.14	<0.04	<0.04	1.57
BDNF		65.55	76.47	54.58	24.67	32.15	43.08
TRAIL		59.49	7.13	11.02	18.07	6.56	160.19
G-CSF		60.44	29.13	27.61	29.19	22.93	46.9
GROa		<1.32	<1.32	<1.32	<1.32	<1.32	2.53
IL-1a		<0.03	<0.03	<0.03	<0.03	<0.03	2.02
IL-23		<1.14	<1.14	<1.14	<1.14	<1.14	280.22
IL-12P40		12.28	5.28	5.51	5.91	4.85	5.03
IL-15		23.26	15.04	15.2	15.5	8.87	21.87
IL-18		63.32	32.75	29.23	20.58	15.88	57.87
M-CSF		<21.26	<21.26	<21.26	<21.26	<21.26	66.35
MCP-3		34.09	19.77	21.77	22.66	15.27	36.8
MIG		116.94	48.44	43.24	51.03	29.88	90.65
RESISTIN		2169.11	882.64	883.12	1044.29	1040.78	1797.87
IL-21		<6.86	<6.86	<6.86	<6.86	<6.86	51.06
sICAM-1		2873.88	1288.51	1352.51	1511.39	1383.53	438.41
sVCAM-1		18321.17	14333.65	21242.87	27445.4	32069.94	24312.58
FGF-2		<4.96	<4.96	<4.96	<4.96	<4.96	46.07
IL-22		197.41	88.68	51.37	137.2	15.4	187.74
PDGF-BB		152.83	106.79	67.24	24.24	29.07	131.28
VEGF-A		86.41	18.9	22.79	17.07	12.28	111.3
LEPTIN		1168.41	564.41	490.06	382.1	276.88	1555.23
PAI-1		3267.75	3342.01	3740.43	3166.38	4289.22	2968.45
sCD40L		29.66	11	9.13	16.18	9.6	36.21
ENA78		<8.06	<8.06	<8.06	<8.06	<8.06	71.33

The numbers presented in the table are the detected protein absolute levels in pg/ml for days 2, 4, 9, 17 and 34. Pre-vaccination plasma was unavailable, thus the day 34 levels may serve as a best available baseline. Also, plasma from an irrelevant normal control was evaluated (the far right column, blue highlight). Some of these plasma proteins may have been down regulated in response to the vaccine, but this is unknown since pre-vaccination plasma was unavailable.

eFigure. Gating strategies



Representative dot plots from cryopreserved peripheral blood mononuclear cells showing gating strategies to identify: (A) activated (HLA-$DR^{+}CD38^{+}$) T cells. Single cells were selected for analysis on the basis of forward scatter area and height characteristics (FSC-A and FSC-H). Live lymphocytes were then identified by FSC-A and side scatter area (SSC-A) gating followed by Zombie Aqua dye exclusion. $CD3^{+}CD4^{+}CD8^{-}$ cells and $CD3^{+}CD4^{-}CD8^{+}$ cells were defined as CD4 and CD8 T cells, separately; and (B) plasmablasts. After live lymphocytes were selected as described above, total B cells were defined as both CD19- and/or $CD20^{-}$expressing cells following CD3 (T lymphocytes) and CD14 (monocytes) exclusion. Plasmablasts were then identified as CD27hi/CD38hi cells. The red boxes represent the gates used to capture each cell sub-population.